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Application of thiophanate-methyl at different host growth stages for management of sclerotinia stem rot in soybean

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Abstract

Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, is an important disease of soybean (*Glycine max* L.) in the North Central United States. The incidence of SSR can be reduced by planting partially resistant cultivars and by implementation of cultural practices that limit pathogen activity. Fungicides such as thiophanate-methyl are another option for control of SSR, but usually recommended in situations where susceptible cultivars must be grown or modification of cultural practices are not disease control options. Previous studies have shown that control of SSR with fungicides is possible, but that the degree of control is inconsistent especially when incidence of SSR surpasses 50%. This study was designed to evaluate the efficacy of single or multiple applications of thiophanate-methyl in relation to timing of inoculation and early reproductive growth stages of the host. Inoculum was introduced at growth stage R2 at the Illinois location, and naturally occurring inoculum was relied upon to initiate SSR at the Wisconsin location. Thiophanate-methyl was effective when applied at R1 prior to inoculation at R2, but not if applied at R3, after inoculation. Two applications of thiophanate-methyl starting at R1 or the R2 growth stage and a second 2 weeks later lowered the incidence of SSR at the naturally infested Wisconsin site compared to one application with one exception. The incidence of SSR was identical for one and two applications starting at R1.5. Soybean yield was improved by fungicides at both experimental locations, and coincided with timing, and number of applications. Data from both locations suggested that fungicides need to be applied prior to inoculum arrival to the infection court. One application was effective in the controlled inoculation study, but two applications were needed at the naturally infested location. Two applications of thiophanate-methyl improved yield of a partially resistant soybean cultivar even though the incidence of SSR was $<10\%$ at the Wisconsin location. Thiophanate-methyl is an effective option for control of SSR, especially to reduce seedborne inoculum in commercial seed production systems.

Keywords: Sclerotinia stem rot; Fungicide; Timing; Thiophanate-methyl; Management

1. Introduction

Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, has emerged as an important disease of soybean in the North Central United States (Workneh and Yang, 2000; Wrather et al., 2001). Prior to 1992, SSR was considered of minor importance in the North Central Region and usually occurred only in Michigan, Minnesota, North Dakota, northern Illinois, and Wisconsin (Grau, 1988; Nelson et al., 1991). Localized outbreaks of SSR usually occurred when other susceptible crops were grown in rotation with soybean. Beginning in 1992, however, SSR outbreaks occurred in Illinois, Indiana, Iowa, Nebraska, Ohio, and Pennsylvania as well as the traditional states of Michigan, Minnesota, North Dakota and Wisconsin (Workneh and Yang, 2000; Wrather et al., 2001, 2003).

The widespread occurrence of SSR has been attributed to changes in management practices, susceptible germplasm, and favorable weather conditions. The use of narrower row spacing, higher plant populations, and optimal fertilizer application create a dense plant

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canopy that is conducive to high humidity and cooler temperature conditions favoring fungal infection and disease development. Shortened crop sequences usually consisting of soybean and other susceptible crops result in the accumulation of soilborne inoculum. Reduced tillage or no-till systems allow soilborne inoculum to accumulate on or near the soil surface, increasing apothecia formation and the release of the airborne ascospores. Spread of *S. sclerotiorum* inoculum in infected seed has probably contributed to the spread of the disease (Hartman et al., 1998; Hoffman et al., 1998; Mueller et al., 1999). Also contributing to the increased occurrence of SSR is the use of susceptible germplasm as the parent material for many soybean cultivars planted in the North Central Region.

The incidence of SSR can be reduced by planting partially resistant cultivars and by altering cultural practices that favor pathogen activity (Kim et al., 2000; Kurle et al., 2001; Gracia-Garza et al., 2002; Mueller et al., 2002b). However, when suitable environmental conditions are present, damaging outbreaks of SSR can still occur. Crop protection chemicals are a control option when this situation occurs, where susceptible cultivars must be grown, or where modification of cultural practices is not an option to reduce the risk of SSR.

Crop protection chemicals used to control SSR have included herbicides and fungicides. The postemergence herbicide, lactofen, has received some attention as a potential chemical agent for controlling SSR (Dann et al., 1999; Nelson et al., 2002). Previous studies have identified fungicides that are active against *S. sclerotiorum*. Such fungicides provide control of SSR in the field, but lack consistency when incidence of SSR surpasses 50% (Mueller et al., 2002a). Although several fungicides have proven efficacy in controlling SSR, thiophanate-methyl is currently the only fungicide registered for control of SSR on soybean in the US.

Because flower petals are the primary infection court, timing of fungicide applications must be coordinated with crop growth and development to achieve control of SSR in soybean. Sclerotia of *S. sclerotiorum* function as survival structures and germinate to form apothecia whenever soil conditions are moist and the crop canopy shades the soil surface (Schwartz and Steadman, 1978; Sun and Yang, 2000). Ascospores are discharged from the apothecia, deposited on petals, germinate, and form mycelia that colonize petals, especially those that adhere to small and recently emerged pods on the lower nodes (Abawi and Grogan, 1975; Sutton and Deverall, 1983; Boland and Hall, 1988; Grau, 1988). The pathogen requires an energy source to support infection of host tissues and petals serve this role in initial stages of infection (Abawi and Grogan, 1975; Sutton and Deverall, 1983). Thus, it is essential that the application of fungicides be timed and directed to obtain complete coverage of flower petals, especially those located on the lower nodes of plants, to achieve control of SSR by this tactic. Currently reliable information is lacking on the number and timing of fungicide applications necessary to control SSR in soybean.

Most research on fungicides for control of *S. sclerotiorum* has focused on various types of common bean (*Phaseolus vulgaris* L.). Data and recommendations for fungicide usage on snap and dry bean can conceivably be used as guidelines for soybean (Hunter et al., 1978; Steadman, 1983; Morton and Hall, 1989). However, there are enough fundamental differences in growth habit between common bean and soybean to justify additional research into the application of fungicides to soybean for control of SSR. Edible bean has a determinate growth habit and produces flowers for approximately 2 weeks (Steadman, 1983). Soybean cultivars grown in the North Central region have indeterminate stem growth and flowers develop at new nodes over a 4-week time period (Fehr et al., 1971). Thus, petals are present longer and provide an infection court for a much longer period of time compared to edible bean types. Another difference relevant to the effective use of fungicides for SSR control in soybean is that flower petals of common bean dislodge from flowers and are scattered within the crop canopy. Soybean flower petals, in contrast, usually remain attached to emerging pods (Grau, 1988). These differences in flower development and petal retention are a partial explanation why symptoms and signs are distributed throughout the plant in edible bean (Hall and Steadman, 1991), while they are concentrated at nodes and stem internodes in soybean (Grau and Hartman, 1999).

The objective of this study was to determine the optimum number and timing of fungicide applications necessary for control of SSR in soybean.

2. Materials and methods

Field experiments were conducted in Illinois and Wisconsin to determine the optimum number and timing of thiophanate-methyl applications necessary to control SSR. Plants in Illinois experiments were inoculated with autoclaved wheat (*Triticum aestivum* L.) kernels colonized by *S. sclerotiorum*. Plots in Wisconsin relied on natural inoculum of *S. sclerotiorum*.

2.1. Illinois location

Dwight, an SSR susceptible soybean cultivar, was planted on 25 May 2000 and 23 May 2001 in an irrigated field near Urbana, IL with no history of SSR. The soil type was Drummer silt loam. Tillage consisted of field cultivation in the spring prior to planting. An

irrigation system was set to mist 30 s every 15 min throughout the day and 1 min every hour throughout the night. Rain Bird (Glendora, CA) 10F nozzles were placed every 4.5 m on risers extending 1.2 m above the soil surface to ensure uniform coverage. Plots consisted of six rows 4 m in length with a 38 cm spacing between rows planted at a density of 544,000 seeds/ha. Inoculum of a highly aggressive isolate of *S. sclerotiorum* (SSR-113) from soybean was grown on autoclaved wheat grain using a method described previously (Mueller et al., 2002a). All plants in each treatment plot were inoculated with 50 g of the wheat-based inoculum. Inoculum was applied by hand to top of the soybean canopy at approximately the R2 growth stage (Fehr et al., 1971) on 18 July 2000 and 10 July 2001.

Thiophanate-methyl was applied with a hand-held CO_2 pressurized sprayer calibrated to deliver 238 l/ha at 173 kPa pressure using XR8003VS flat fan nozzles (Spraying Systems Co., Wheaton, IL) at approximately 5.0 km/h. In 2000, applications were made on 14 July (R1 reproductive growth stage, before inoculation) or 27 July 2000 (R3 reproductive growth stage, after inoculation). In 2001, applications were made on 7 July (R1 reproductive growth stage, before inoculation) or 16 July 2001 (R3 reproductive growth stage, after inoculation). Treatments were a non-treated control and two rates of thiophanate-methyl, 0.56 or 1.12 kg a.i./ha. The treatments were arranged in a split-plot design with four replications and fungicide timing (application before or after inoculation) as the main split.

A disease severity index (DSI) (Grau, 1988) was used to estimate the severity of SSR in each treatment plot. The DSI ratings were taken at reproductive growth stage R7 (Fehr et al., 1971). The DSI was determined by rating disease severity of 30 random plants from the center rows of plots using a scale of 0–3 where 0 = no symptoms, 1 = lesions only found on lateral branches, 2 = small lesions on main stem not affecting pod fill, and 3 = lesions on main stem resulting in plant death and poor pod fill. The DSI for each plot was calculated by: DSI = [3(rating of each plant)/(3 * number plants rated)] * 100. The DSI ranged from 0 when no plants were diseased to 100 when all rated plants were dead. Plots were harvested with a small plot combine and grain yields were adjusted to 13% moisture. Harvested grain was subsampled for sclerotia mixed with grain during the harvest operation. The number and weight of sclerotia were determined per 100 g of harvested seed.

Data were analyzed using the GLM procedure of the Statistical Analysis System (SAS) (SAS Institute, Cary, NC) to perform an analysis of variance (ANOVA). Means were compared using Fisher's protected least significant difference test at $p = 0.05$. Sclerotia number and weight from non-treated plots were compared to all fungicide-treated plots in single-degree-of-freedom contrasts.

2.2. Wisconsin (naturally infested field site)

Thiophanate-methyl was evaluated for control of SSR at a naturally infested field near Arlington, WI in 1997. Soil type at the site was a Clarion silt loam. Tillage consisted of field cultivation in the fall after harvest and again in the spring prior to planting. Syngenta NK S19-90 (partially resistant to SSR) was planted on 21 May in nine row plots 8.6 m in length with 38 cm spacing between rows at a density of 556,000 seeds/ha. Thiophanate-methyl was applied at two application rates, 0.84 kg a.i./ha, 75% labeled rate and 1.12 kg a.i./ha, a full labeled rate, in a single application at reproductive growth stages R1, R1.25, R1.5 and R2 alone, or in paired applications consisting of a first application at reproductive growth stages R1, R1.25, R1.5 and R2 followed by a second application 2 weeks later. Fungicide was applied using a plot sprayer mounted on a tractor (IH Cub, Case International, Racine, WI). The system was calibrated to deliver 280 l/ha at 241 kPa pressure using XR80015VS flat-fan nozzles (Spraying Systems Co.). Plots were arranged in a randomized complete block design with four replications. Incidence of SSR was recorded at reproductive growth stage R7 by counting 100 plants in each plot and recording the number plants killed by *S. sclerotiorum*. After end-trimming to 7.6 m, the center four rows of plots were harvested with an Almaco plot combine (Allen Machine Co., Nevada, IA) on 7 October and yields were adjusted to 13% moisture. Data were analyzed using the RCB procedure of MSTAT (Michigan State University) to perform an ANOVA. Mean separation was performed using Fisher's protected least significant difference test at $p = 0.05$.

3. Results

3.1. Illinois (inoculated plots)

There was a significant year by treatment interaction for DSI and yield so data from 2000 and 2001 were not combined. There was no significant year by treatment interaction for sclerotia number or weight so data for these variables was combined. The mean DSI of SSR for control plots ranged from 42 to 49 in both years of the study thus providing relatively high levels of disease development for comparison of fungicide treatments. The mean yield for all treatments was 4145 and 3028 kg/ha for 2000 and 2001, respectively (Table 1). Fungicides applied at reproductive growth stage R1 prior to the arrival of inoculum reduced SSR severity both years. In 2000, the severity of SSR was lowered and yield increased in plots treated with both the 0.56 kg a.i./ha and the 1.12 kg a.i./ha rates of thiophanate-methyl applied prior to inoculation when compared to plots

Table 1
Effect of time and rate of thiophanate-methyl application in relation to inoculation[a] for control of Sclerotinia stem rot and yield of cultivar Dwight in an inoculated, irrigated field in Illinois, 2000 and 2001

Treatment	Timing	Rate (kg a.i./ha)	2000		2001	
			DSI[b]	Yield (kg/ha)	DSI[b,c]	Yield (kg/ha)[c]
Non-treated control	—	—	42.3	3734	48.9 c	2711 b
Thiophanate-methyl	R1	0.56	6.1	4595	37.2 b	3169 ab
Thiophanate-methyl	R1	1.12	2.5	4865	15.3 a	3425 a
Thiophanate-methyl	R3	0.56	43.6	3593	39.5 b	2839 b
Thiophanate-methyl	R3	1.12	36.1	3929	50.3 c	3008 ab
Mean			26.1	4145	38.2	3028
LSD ($p=0.05$)			16.5	468	20.8	476

[a] Inoculum was applied at approximately the R2 reproductive growth stage.
[b] Disease severity index (DSI) estimated at R7 reproductive growth stage and calculated by: DSI = [3 (rating of each plant)/3 * number plants rated] * 100.
[c] Means followed by identical letter are not statistically different according to Fisher's protected least significant difference when $p=0.05$.

not treated with the fungicide. In 2001, a high rate of thiophanate-methyl was required for both control of SSR and an increase in yield. When thiophanate-methyl was applied after inoculation, there was no effect on SSR severity or yield in either year of the study. Significantly more sclerotia were mixed with seed harvested from non-treated plots (6.0 sclerotia/100 g seed) compared to plots treated with both rates of the fungicide (3.2 sclerotia/100 g seed), but there was no difference between rates of fungicides. The weight of sclerotia mixed with seed harvested from non-treated plots (0.1 g sclerotia/100 g seed) was significantly higher than sclerotia weight from seed harvested from fungicide treated plots (0.05 g sclerotia/100 g seed).

3.2. Wisconsin (naturally infested field site)

The mean incidence of SSR was 5% at this location and reached 7% in plots not treated with thiophanate-methyl. Application of thiophanate-methyl significantly lowered plant mortality caused by *S. sclerotiorum* compared to the no fungicide control. However, efficacy was affected by number of applications and growth stage. Two applications of thiophanate-methyl starting at reproductive growth stage R1 or R2 and a second 2 weeks later lowered the incidence of SSR to one application with one exception. The incidence of SSR was identical for one and two applications starting at reproductive growth stage R1.5. Although plant mortality was reduced by two applications of thiophanate-methyl begun at any one of the initial application times, R1, R1.25, R1.5, or R2, only a paired application of thiophanate-methyl begun at R1.25 or R2 increased yield when compared to the non-treated control. Compared to a single application of thiophanate-methyl, yield was increased by 10% if applied twice starting at the R2 reproductive growth stage, and 6% if applied twice starting at the R1.25 reproductive growth stage (Table 2). Yield was improved 8.6% for a comparison of the no fungicide control and yield achieved by two applications of fungicide starting at the R1.25 reproductive growth stage.

4. Discussion

These results confirm the observation of earlier researchers who emphasized the need to protect flower petals from infection if SSR is to be controlled in soybean (Mueller et al., 2002a). Although thiophanate-methyl has localized systemic activity, this fungicide must be applied at the full rate before the arrival of inoculum on flower petals. The highest yields and the lowest disease severity were achieved if plots were treated with a full rate of thiophanate-methyl, applied twice during the flowering growth stages beginning before infection occurred.

Protection of soybean flower petals from infection by *S. sclerotiorum* is difficult because of the indeterminate growth habit of northern soybean cultivars (Fehr et al., 1971). The flowering period of soybean ranges from 1 July to 15 August for adapted soybean cultivars in the North Central Region. The first flowers that appear (R1 growth stage) are usually in the middle of the plant. As the plant grows, most flowers produced are on newly formed nodes at the top of the plant. However, flowers on new branches near the bottom of the plant are also produced. These new flowers at the base of the plant from branches are probably the ones being infected during the final phases of the infection period. The greater effectiveness of two fungicide applications

Table 2
Effect of thiophanate-methyl application on incidence of Sclerotinia stem rot (SSR) and grain yield of the cultivar Syngenta 19–90 in a naturally infested commercial field near Arlington, WI, 1997

Treatment	Timing	Rate (kg a.i./ha)	SSR incidence (%)[a,b]	Yield (kg/ha)[a]
Non-treated control	—	—	7 bc	3431 ab
Thiophanate-methyl	R1 and 2 weeks later	0.84 + 0.84	4 a	3452 ab
Thiophanate-methyl	R1	1.12	9 c	3405 ab
Thiophanate-methyl	R1.25 and 2 weeks later	0.84 + 0.84	2 a	3754 c
Thiophanate-methyl	R1.25	1.12	7 bc	3519 b
Thiophanate-methyl	R1.5 and 2 weeks later	0.84 + 0.84	2 a	3492 b
Thiophanate-methyl	R1.5	1.12	5 ab	3411 a
Thiophanate-methyl	R2 and 2 weeks later	0.84 + 0.84	1 a	3674 bc
Thiophanate-methyl	R2	1.12	7 bc	3310 a
Means			5	3485
LSD ($p = 0.05$)			3	180

[a] Incidence of SSR was recorded at reproductive growth stage R7 by counting 100 plants in each plot and recording the number plants killed by *S. sclerotiorum*.
[b] Means followed by identical letter are not statistically different according to Fisher's protected least significant difference when $p = 0.05$.

suggests that new flowers form on lower nodes after the first application.

Differing degrees of correlation have been reported for the relationship between plant mortality and disease severity, caused by *S. sclerotiorum*, and yield (Grau, 1988; Hoffman et al., 1998; Yang et al., 1999). Yield reduction estimates range from 147 to 335 kg/ha for each 10% increment of plant mortality or disease severity for susceptible cultivars (Grau, 1988; Hoffman et al., 1998; Yang et al., 1999). However, Yang et al. (1999) reported that yield reductions by SSR were insignificant when plant mortality was less than 20%. Data from this current study suggests that yield reduction can occur when the incidence of SSR is undetectable on the stems. It is conceivable that pods are infected and decayed by *S. sclerotiorum* without the pathogen progressing into the stem and causing plant mortality. Advances in breeding for resistance have been to reduce plant mortality by *S. sclerotiorum* (Kim et al., 2000). Results of this study suggest that resistance to pod decay may be the next order of refinement in breeding soybean cultivars for resistance to *S. sclerotiorum*.

Inconsistent control by fungicides of SSR caused by *S. sclerotiorum* is generally considered to be the result of inadequate coverage of the potential infection court prior to deposition of ascospores. Our results support this understanding of control of SSR with fungicides. Early recommendations for use of fungicides to control SSR called for applications when flowers first appear. Our research suggests that fungicides may need to be applied after flowering has begun and a second application may be necessary to reduce incidence and severity of SSR and increase yield.

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